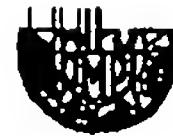


PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C07D 499/20, 501/12		AJ	(11) International Publication Number: WO 99/55710 (13) International Publication Date: 4 November 1999 (04.11.99)
(21) International Application Number: PCT/NL99/00246 (22) International Filing Date: 27 April 1999 (27.04.99) (30) Priority Data: 98201398.9 29 April 1998 (29.04.98) EP		(81) Designated States: AB, AL, AU, BA, BB, BG, BR, CA, CN, CU, CZ, DE, GD, GB, HR, HU, ID, IL, IN, IS, JP, KP, KR, LC, LK, LR, LT, LV, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, SL, TR, TT, UA, US, UZ, VN, YU, ZA, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for all designated States except US): DSM N.V. [NL/NL]; Het Overtoon 1, NL-6411 TE Heerlen (NL).		Published With international search report.	
(72) Inventors; and (73) Inventors/Applicants (for US only): VAN DER DOES, Thomas [NL/NL]; Van der Haerntaat 47, NL-2613 ZA Delft (NL). KUIPERS, Rienk, Hendrik [NL/NL]; Waterhuizen 18, NL-2804 KZ Gouda (NL).			
(74) Agent: JACOBS, Monique, Sophie, Nicole; Octroobureau DSM, I-1000, NL-6160 MA Geleen (NL)			
(54) Title: A METHOD FOR CRYSTALLIZING A β -LACTAM ANTIBIOTIC			
(57) Abstract The invention relates to a method for crystallizing a β -lactam, wherein the β -lactam is crystallized from a nitric acid solution.			

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Angola	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SL	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	MN	Republic of Macedonia	TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Mauritius	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KC	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's	NZ	New Zealand		
CR	Cameroon	KR	Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LJ	Liechtenstein	SD	Sierra Leone		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
DO	Dominican Republic	LR	Liberia	SG	Singapore		

A METHOD FOR CRYSTALLIZING A β -LACTAM ANTIBIOTIC

5 The invention relates to a method for crystallizing a β -lactam and to a β -lactam obtainable by said method.

The term β -lactam as used herein includes β -lactam nuclei, for example 6-aminopenicillanic acid (6-APA), 7-aminocephalosporanic acid (7-ACA), 3-chloro-7-aminodesacetoxydesmethylcephalosporanic acid (7-ACCA), and 7-amino-3-[(1-methyl-1-H-tetrazol-5-yl)thio]methyl-3-cephem-4-carboxylic acid (7-ATCA), 7-aminodesacetylcephalosporanic acid (7-ADAC), and 7-aminodesacetoxycephalosporanic acid (7-ADCA), fermentation products, for example penicillin G, penicillin V, cephalosporin C, isopenicillin N, intermediate products for example adipyl-6-aminopenicillanic acid, adipyl-7-aminodesacetoxycephalosporanic acid (adipyl-7-ADCA), adipyl-7-aminocephalosporanic acid (adipyl-7-ACA), adipyl-7-aminodesacetylcephalosporanic acid (adipyl-7-ADAC), 3-carboxyethylthiopropionyl-7-aminodesacetoxycephalosporanic acid, 2-carboxylethylthioacetyl-7-aminodesacetoxycephalosporanic acid and 3-carboxyethylthiopropionyl-7-aminodesacetoxycephalosporanic acid, and β -lactam antibiotics, for example ampicillin, amoxicillin, cephalexin, cephadrine, cefprozil, cefaclor and cefadroxil.

30 The last few decades, β -lactams have received a lot of attention, because many compounds of this class show antimicrobial activity. In particular, the β -lactam antibiotics, for example penicillin and cephalosporin antibiotics, are useful because of their antimicrobial activity and play an important role in

medicine. This class of antibiotics comprises a great variety of compounds, all having their own activity profile. In general, β -lactam antibiotics consist of a nucleus, the so-called β -lactam nucleus, which is 5 linked through its primary amino group to the so-called side chain via a linear amide bond.

Recently, many semi-synthetic routes to β -lactam antibiotics have been reported. According to those semi-synthetic routes, the synthesis of a β -10 lactam antibiotic generally comprises the preparation of a β -lactam nucleus from fermentation products for example isopenicillin N, penicillin G, penicillin V and cephalosporin C. The obtained β -lactam nucleus is subsequently attached to one of several possible side 15 chains to obtain the antibiotic product.

These semi-synthetic routes involve enzymatic catalysis, which leads to highly selective and clean preparation processes. In contrast with the conventional chemical processes, enzymatically 20 catalyzed reactions may be performed in aqueous environment and generate hardly any by-products, if at all. Also, it is usually not necessary to perform any protection and deprotection steps, which are so often imperative in organic synthesis, in these enzymatically 25 catalyzed processes.

Although the semi-synthetic routes produce significantly less by-products compared to the conventional processes, the intermediate product and the final product, i.e. the β -lactam nucleus and the β -30 lactam antibiotic, still need to be isolated from a reaction mixture and purified. Usually, the β -lactam is isolated from a reaction mixture and purified by crystallization in a procedure which is essentially the same as the procedure that would be performed if the

product were obtained in a conventional synthetic process.

A typical example of such a crystallization process is described in the British patent application 1 500 236. This document discloses a process wherein 6-aminopenicillanic acid is acylated with α -aminophenylacetic acid chloride, HCl, in aqueous acetone. The final β -lactam antibiotic is isolated by crystallization from a hydrochloric acid solution by 10 addition of a suitable base, for example NaOH or ammonia.

The conventional crystallization processes start from a hydrochloric acid solution of the β -lactam, from which the product is crystallized by 15 addition of an alkaline solution, usually an NaOH solution. It has been found that the yield of these conventional crystallization processes is rather low. This is most likely due to a significant loss of product to the mother liquor.

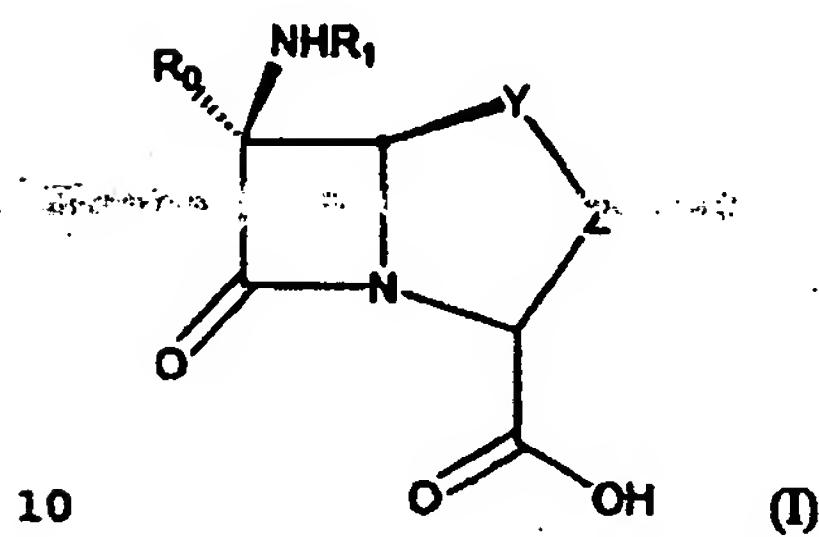
20 Surprisingly, it has now been found, that the yield of a crystallization process of a β -lactam can be increased by starting from a solution of the β -lactam in nitric acid. Accordingly, the invention provides a method for crystallizing a β -lactam, wherein 25 the β -lactam is crystallized from a nitric acid solution.

Besides the significant improvement in yield of product of a method according to the invention, a great advantage of the invention is that 30 the volumetric throughput of a large scale production process of β -lactam is increased. It has been found that, when the β -lactam is crystallized from a nitric acid solution, it is feasible to perform the

- 4 -

crystallization process using higher concentrations of β -lactam than hitherto have been thought possible. As a result, less reactor volume is needed in order to obtain an equal amount of β -lactam.

5 A β -lactam that may be crystallized in a method according to the invention preferably has the general formula (I):



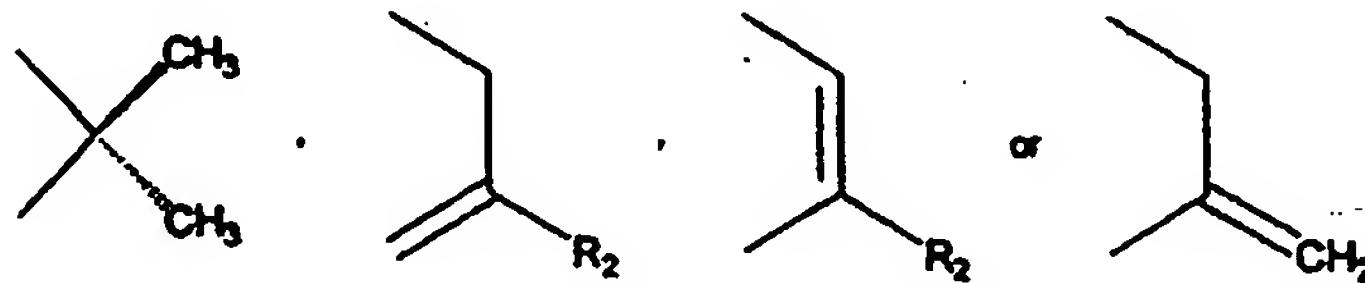
wherein

R0 is hydrogen or C₁₋₃ alkoxy;

R1 is hydrogen or a side chain derived from an organic acid;

Y is CH₂, oxygen, sulfur, or an oxidized form of sulfur; and

Z is



20

wherein R₂ is hydrogen, hydroxy, halogen, C₁₋₃ alkoxy, optionally substituted, optionally containing one or more heteroatoms, saturated or unsaturated, branched or straight C₁₋₃ alkyl, optionally substituted, optionally containing one or more heteroatoms, C₅₋₈ cycloalkyl,

- 5 -

optionally substituted aryl or heteroaryl, or optionally substituted benzyl. Preferably, R₂ is -H, -Cl, -OH, -OCH₃, -CH₂OH, -CH₂Cl or -CH₂OC(O)CH₃.

Formula (I) is intended to encompass all β -lactams as disclosed in "Cephalosporins and Penicillins, Chemistry and Biology", Ed. E.H. Flynn, Academic Press, 1972, pages 151-166, and "The Organic Chemistry of β -Lactams", Ed. G.I. Georg, VCH, 1992, pages 89-96, which are incorporated herein by reference.

In the context of the invention, an oxidized form of sulfur is meant to include groups for example sulfoxide and sulfone. By optionally substituted alkyl, cycloalkyl, aryl, heteroaryl and benzyl, groups are intended, which have substituents for example alkyl groups of from 1 to 3 carbon atoms.

Especially preferred β -lactams to be crystallized in a method according to the invention are β -lactam antibiotics comprising a β -lactam nucleus coupled to a side chain. These preferred β -lactams are those having formula (I) wherein R₁ is a side chain.

Preferred side chains coupled to a β -lactam nucleus in a β -lactam antibiotic to be crystallized in a method according to the invention are D-(-)-phenylglycine, D-(-)-4-hydroxyphenylglycine, D-(-)-2,5-dihydrophenylglycine, 2-thienylacetic acid, 2-(2-amino-4-thiazolyl)-2-methoxyiminoacetic acid, α -(4-pyridyl-thio)acetic acid, 3-thiophenemalonic acid, 2-cyanoacetic acid, D-mandelic acid, 1H-tetrazoleacetic acid, 2-furanyl-(Z)-methoxyiminoacetic acid, (2-aminothiazol-4-yl)acetic acid, (2-aminothiazol-4-yl)-(2)-hydroxyiminoacetic acid, (2-aminothiazol-4-yl)-(Z)-carboxymethoxyiminoacetic acid, (2-aminothiazol-4-yl)-

- 6 -

(Z)- (1-carboxy-1-methylethoxy) iminoacetic acid or derivatives thereof.

The most preferred β -lactams to be crystallized in accordance with the invention are 5 amoxicillin, ampicillin, cephalexin, cefaclor, cefadroxil, cephadrine, epicillin, cefamandole, cefotaxime, cefdinir, cefprozil, cefuroxim, cefepime, cefibuten, and loracarbef.

In one embodiment, the β -lactam to be 10 crystallized is obtained synthetically. In a synthetic preparation of a β -lactam antibiotic, a β -lactam nucleus, for example 6-APA, 7-ADCA, 7-ACA, 7-ATCA or 7-ADAC, or a derivative thereof is acylated, e.g. according to the so-called Dane process. In this 15 process, the acylation is carried out with a Dane salt of a precursor for the desired side chain, e.g. a Dane salt of phenyl glycine. A Dane salt may be prepared by protecting the amine group of the precursor for the side chain as an enamine, and reacting the product 20 thereof with a reactive acid to form a mixed anhydride. The Dane process has been described in, among others, US-A-4,358,588 and EP-A-0 439 096.

After the acylation of the β -lactam nucleus with the Dane salt has been completed, the amine group 25 has to be deprotected. The deprotection reaction is usually an acidic hydrolysis wherein the protective group is split off. When the β -lactam to be crystallized in accordance with the invention has been prepared in a Dane process, the deprotection step can be 30 advantageously be carried out *in situ* by using nitric acid to facilitate the acidic hydrolysis.

In another preferred embodiment, the β -lactam to be crystallized is obtained enzymatically. When a β -lactam nucleus is to be crystallized, it can

for instance be obtained in a procedure as disclosed in EP-A-0 532 341.

When a β -lactam antibiotic is to be crystallized, it is preferably obtained by enzymatic acylation. This means, that a suitable β -lactam nucleus or a salt thereof is reacted with a suitable precursor for a side chain in the presence of a suitable enzyme, for example a penicillin acylase. Enzymes may be isolated from various naturally occurring micro 10 organisms, for example fungi and bacteria. Organisms that have been found to produce penicillin acylase are, for example, *Acetobacter*, *Aeromonas*, *Alcaligenes*, *Aphanocladium*, *Bacillus* sp., *Cephalosporium*, *Escherichia*, *Flavobacterium*, *Kluyvera*, *Mycoplana*, 15 *Protaminobacter*, *Pseudomonas* or *Xanthomonas* species.

Of course, it is possible to use the enzyme as the free enzyme or in any suitable immobilized form. In addition, it is possible to use functional equivalents of the enzyme, wherein for instance 20 properties of the enzyme, for example pH dependence, thermostability or specific activity may be affected by chemical modification or cross-linking. Also, functional equivalents for example mutants or other derivatives, obtained by classic means or via 25 recombinant DNA methodology, biologically active parts or hybrids of the enzymes may be used.

Suitable salts of a β -lactam nucleus in this regard include any non-toxic salt, for example an alkali metal salt (e.g. lithium, potassium, sodium), an 30 alkali earth metal salt (e.g. calcium, magnesium), an ammonium salt, or an organic base salt (e.g. trimethylamine, triethylamine, pyridine, picoline, dicyclohexylamine, *N,N'*-dibenzyl diethylene diamine).

The precursor for a side chain of the β -

lactam antibiotic to be prepared in a method according to the invention may be any compound that is recognized by the above defined enzyme, for example penicillin acylase, and that leads to a product of the class of β -lactam antibiotics. It is possible to use the compound corresponding to the side chain in itself, but also derivatives thereof may be used. Suitable derivatives of these compounds are esters and amides, wherein the side chain molecule is connected to a C₁-C₃ alkyl group through an ester or amide linkage.

After the enzymatic acylation of a β -lactam nucleus in a preparation of a β -lactam antibiotic as described hereinabove, the enzyme is separated from the reaction mixture. This may for instance be done by filtration in case an enzyme is used in immobilized form. After separation of the enzyme, the thus obtained reaction mixture may be used as such in a method according to the invention or it may be further treated.

Of course, it is also possible to combine the above described synthetic and enzymatic preparations of the β -lactam to be crystallized in accordance with the invention.

In a method according to the invention, the β -lactam starting material to be crystallized is dissolved using an aqueous nitric acid solution. It has been found that optimum results are obtained when the pH of the resulting nitric acid solution, wherein the β -lactam is dissolved, is between about 0.3 and about 2.0, preferably between about 0.5 and about 1.5. The concentration of the aqueous nitric acid solution to be added to the β -lactam starting material is preferably between 0.5 mol/liter and 11 mol/liter, more preferably between 5 and 10 mol/liter. It is also possible to use

mixtures of different acids, preferably of different strong inorganic acids, resulting in a pH of the solution, wherein the β -lactam is dissolved, within the above ranges. However, it is desired that the

5 concentration of nitrate ions in the mixture wherein the β -lactam is present is at least 0.3 mol/liter. It is within the expertise of the skilled person to chose the amount of the inorganic acid other than nitric acid such that no (addition) salts of the β -lactam will be

10 formed.

In a preferred embodiment of the invention, the β -lactam is crystallized from a nitric acid solution, in which said β -lactam is present in a very high concentration. In the conventional crystallization processes, the concentration of the β -lactam in the hydrochloric acid solution from which it is crystallized is generally about 0.35 moles/liter. It has now been found that an increased concentration of the β -lactam in the solution from which it is

15 crystallized leads to a higher yield of the crystallization process. In this text yield is defined as moles of isolated crystal per moles of β -lactam starting material. An increase in yield has been proved to be achievable in a process according to the

20 invention, which corresponds to a decrease in loss of the desired β -lactam to the mother liquor during the crystallization of 25-50%. In addition, a much higher volumetric throughput may be achieved when a crystallization process is started from a solution

25 wherein a β -lactam is present in a high concentration.

A high concentration β -lactam in the nitric acid solution from which it is crystallized in accordance with this preferred embodiment is higher

- 10 -

than about 0.4 moles/liter. More preferably, the β -lactam is crystallized from a nitric acid solution wherein it is present in a concentration of more than about 0.5 moles/liter. Most preferably, said 5 concentration is higher than about 0.6 moles/liter.

There is no upper limit for the concentration of the β -lactam in the nitric acid solution from which it is crystallized. However, it will be evident that a concentration that is so high 10 that crystallization of the β -lactam in the nitric acid solution starts spontaneously is not suitable.

From the nitric acid solution, the β -lactam is preferably crystallized by the addition of an alkaline solution. Particularly suited for this purpose 15 are ammonia or hydroxide salt solutions. It is preferred that the hydroxide salt is an ammonium or alkali metal salt. The concentration of the alkaline solution will generally lie between about 0.5 and about 8 moles/liter. Preferably, said concentration lies 20 between about 1.5 and about 2.5 moles/liter.

The temperature, at which the method of the invention is performed, will generally lie between about -5°C and 50°C. Preferably, the temperature will lie between about 0°C and 15°C.

25 Upon addition of the alkaline solution, the β -lactam will crystallize. Subsequently, the obtained β -lactam crystals are filtered off and dried in any suitable manner.

In a preferred embodiment, the method of 30 the invention is performed continuously. The advantages of this embodiment will be apparent to the skilled person and include short residence times, small losses of desired product due to a decrease in decomposition, and the possibility of using small installations

- 11 -

resulting in a decrease in costs. Preferably, in this embodiment the β -lactam to be crystallized is dissolved into the nitric acid using a static mixer, which leads to a particularly efficient process.

5 The invention will now be elucidated by the following non-restrictive examples.

Comparative Example I

10 Recrystallization of Amoxicillin trihydrate using hydrochloric acid

At 20°C, Amoxicillin trihydrate (122 g) was suspended in water (500 ml) and concentrated 12 M hydrochloric acid (40 ml) was added to give a pH of 15 0.7. In order to dissolve all material, water (1600 ml) was added. Amoxicillin trihydrate was crystallized by adding a 2M solution of sodium hydroxide in water until a pH value of 5.0 was reached. The crystals thus produced were isolated by means of filtration, washed 20 with water (200 ml) and dried at 35°C during 16 h to give 123 g of Amoxicillin trihydrate. The mother liquor (2.62 l) contained 8.5 g of dissolved Amoxicillin trihydrate.

25 Example I

Recrystallization of Amoxicillin trihydrate using nitric acid

At 20°C, Amoxicillin trihydrate (133 g) was suspended in water (500 ml) and 8M solution of nitric acid in water (60 ml) was added to give a pH of 0.7. All material was dissolved. Amoxicillin trihydrate was crystallized by adding a 2M solution of sodium hydroxide in water until a pH value of 5.0 was reached. The crystals thus produced were isolated by means of

filtration, washed with water (200 ml) and dried at 35°C during 16 h to give 133 g of Amoxicillin trihydrate. The mother liquor (0.68 l) contained 3.1 g of dissolved Amoxicillin trihydrate.

5

Comparative Example II (PRIVATE)

Recrystallization of Cefaclor monohydrate using sulfuric acid

10 At 20°C, Cefaclor monohydrate (11.0 g) was suspended in water (55 ml) and 9.4 M sulfuric acid (7.3 g) was added to give a pH of 1.0. In order to dissolve all material, water (106 ml) is added while the pH is maintained at 1.0 using 9.4 M sulfuric acid (14.3 g).

15 Cefaclor monohydrate was crystallized by adding a 25% solution of ammonia in water (8.9 ml) until a pH value of 6.2 was reached. The crystals thus produced were isolated by means of filtration, washed with water (15 ml) and dried for 16 h at 20°C under vacuum to give 8.2

20 g of Cefaclor monohydrate. The mother liquor (198 g) contained 2.7 g of dissolved Cefaclor monohydrate.

Example II

Recrystallization of Cefaclor monohydrate using nitric acid

25 At 20°C, Cefaclor monohydrate (11.0 g) was suspended in water (55 ml) and 4 M nitric acid (8.1 g) was added to give a pH of 1.0. In order to dissolve all material, water (31 ml) is added while the pH is maintained at 1.0 using 4 M nitric acid (2.5 g).

30 Cefaclor monohydrate was crystallized by adding a 25% solution of ammonia in water (3.8 ml) until a pH value of 6.2 was reached. The crystals thus produced were isolated by means of filtration, washed with water (15

ml) and dried for 16 h at 20°C under vacuum to give 8.8 g of Cefaclor monohydrate. The mother liquor (110 g) contained 2.2 g of dissolved Cefaclor monohydrate.

5

Example III

Crystallization of crude amoxicillin using nitric acid

A wet cake obtained by an enzymatic condensation as described in e.g. WO-A-92/01061 (total weight 3 kg), containing amoxicillin trihydrate (1451 g), D-(-)-hydroxyphenylglycin (HPG, 134 g) and insolubles, was suspended by addition of water, to a total volume of 5 liters. The mixture was cooled to 2°C. Then 5 ml of a 5% EDTA solution were added to the suspension. The mixture was pumped continuously to a dissolution vessel with a rate of 80 ml/min. The pH in the dissolution vessel was kept at 0.7 by addition of 8M nitric acid. The temperature in the vessel was maintained at 5°C. By continuously removing acidic amoxicillin solution from the reaction vessel, the volume in the vessel was kept at 800 ml. This acidic amoxicillin solution was pumped continuously through a Seitz T500 filter (diameter 10 cm) to remove undissolved impurities. The filtrate was added continuously to a crystallizer.

In this crystallizer, the temperature was maintained at 20°C and the pH was kept at 3.7 with the aid of 2 M sodium hydroxide solution. The volume in the crystallizer was kept at 1800 ml by continuously transferring the contents to a second crystallizer. In the second crystallizer, the temperature was maintained at 20°C and the pH was kept at 5.0 with the aid of 2 M sodium hydroxide solution. The volume in the second crystallizer was kept at 1000 ml by continuously

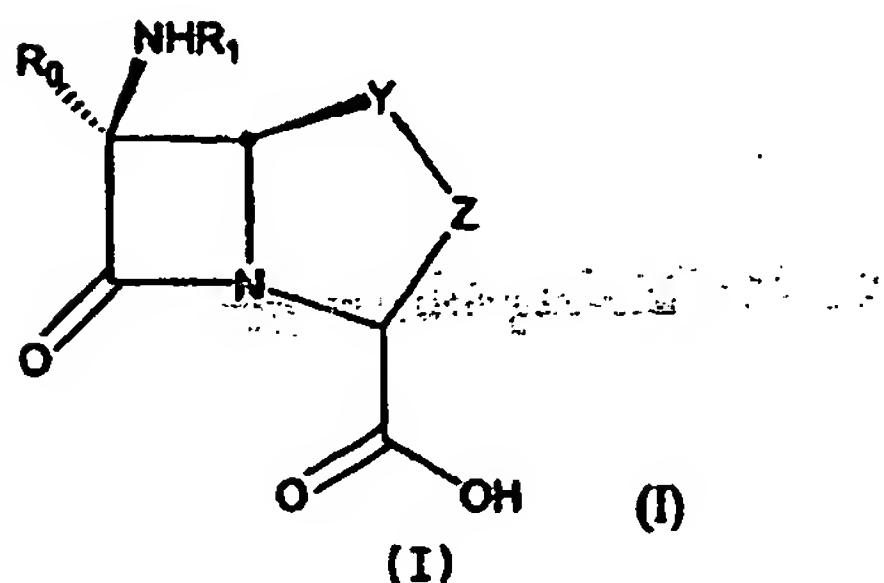
removing the contents to a buffer vessel.

After the addition of suspension to the dissolution was complete, the contents of the dissolution vessel were filtered and added to the first 5 crystallizer, in which the above conditions were maintained. Subsequently, the contents of the first crystallizer were transferred to the second crystallizer, in which the above conditions were maintained, followed by transfer of the contents of the 10 second crystallizer to the buffer vessel.

The total amount of 8 M nitric acid solution consumed was 625 ml. The total amount of 2 M sodium hydroxide solution consumed was 2500 ml. The contents of the buffer were cooled to 2°C and kept at 15 this temperature for more than 2 hours. The resulting crystal suspension was filtered and washed with 1500 ml water. The filter cake was dried in a ventilation stove at 35°C. The final yield of amoxicillin trihydrate (assay 99.5%) (assay is defined here as gram 20 amoxicillin trihydrate per gram crystal * 100%) was 1429 g (98%). The mother liquor contained approximately 26 g (1.8%) amoxicillin trihydrate.

CLAIMS

1. A method for crystallizing a β -lactam, wherein the β -lactam is crystallized from a nitric acid solution.
- 5 2. A method according to claim 1, wherein the pH of the nitric acid solution is between about 0.5 and about 2.0.
3. A method according to claim 1 or 2, wherein the β -lactam is crystallized by addition of an alkaline solution to the nitric acid solution.
- 10 4. A method according to claim 3, wherein the alkaline solution is ammonia or a hydroxide salt solution.
- 15 5. A method according to claim 4, wherein the hydroxide salt is an ammonium or alkali metal salt.
6. A method according to any of the preceding claims, wherein the β -lactam has the general formula (I):
- 20

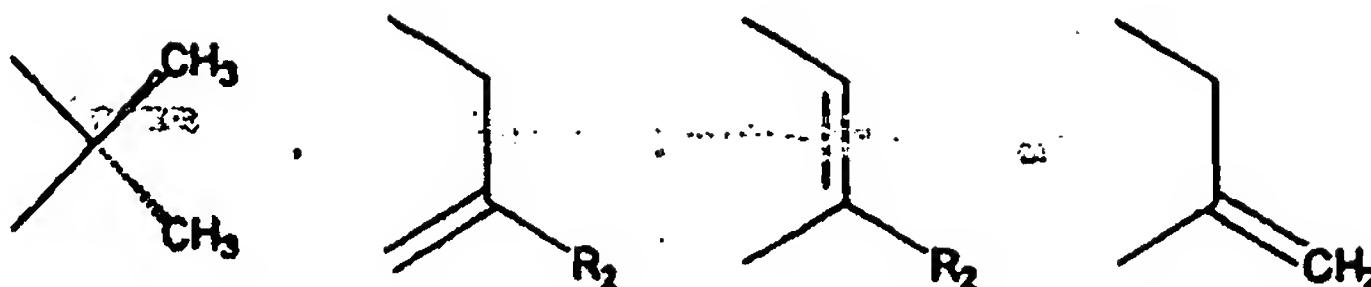


- 25 25. wherein
 - R₁ is hydrogen or C₁₋₁₀ alkoxy;
 - R₁ is hydrogen or a side chain derived from an organic acid;
 - Z is CH₂, oxygen, sulfur, or an oxidized form of sulfur;

- 16 -

and

Z is



5 wherein R₂ is hydrogen, hydroxy, halogen, C₁₋₃ alkoxy, optionally substituted, optionally containing one or more heteroatoms, saturated or unsaturated, branched or straight C₁₋₅ alkyl, optionally substituted, optionally containing one or more heteroatoms, C₅₋₈ cycloalkyl, optionally substituted aryl or heteroaryl, or optionally substituted benzyl.

10 7. A method according to claim 6, wherein the β -lactam is a β -lactam antibiotic, consisting of a β -lactam nucleus, which is linked to a side chain through its primary amino group.

15 8. A method according to claim 7, wherein the side chain is chosen from the group of D-(-)-phenylglycine, D-(-)-4-hydroxyphenylglycine, D-(-)-2,5-dihydrophenylglycine, 2-thienylacetic acid, 2-(2-amino-4-thiazolyl)-2-methoxyiminoacetic acid, α -(4-pyridylthio)acetic acid, 3-thiophenemalonic acid, 2-cyanoacetic acid, D-mandelic acid and derivatives thereof.

20 25 9. A method according to claim 8, wherein the β -lactam antibiotic is chosen from the group consisting of amoxicillin, ampicillin, cephalexin, cefaclor, cefadroxil, cephadrine, epicillin, cefamandole, cefotaxime, cefdinir, cefprozil, cefuroxim, cefepime, cefibuten and loracarbef.

- 17 -

10. A method according to any of the preceding claims, wherein the β -lactam has been obtained enzymatically.
11. A method according to any of the preceding 5 claims, wherein the concentration of β -lactam in the nitric acid solution from which it is crystallized is higher than about 0.4 moles/liter.
12. A method according to claim 11, wherein the 10 concentration of β -lactam in the nitric acid solution from which it is crystallized is higher than about 0.5 moles/liter.
13. A method according to any of the preceding 15 claims, wherein the crystallization is performed continuously.
14. A method according to claim 13, wherein the β -lactam to be crystallized is dissolved into the nitric acid solution by using a static mixer.
15. A β -lactam obtainable by a method according to 20 any of the preceding claims.
16. Use of nitric acid to improve a crystallization process of a β -lactam.

INTERNATIONAL SEARCH REPORT

Int. and Application No

PCT/NL 99/00246

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07D499/20 C07D501/12

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07D C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DATABASE WPI Section Ch, Week 9530 Derwent Publications Ltd., London, GB: Class 802, AN 95-230118 XPO02109764 & RU 2 024 851 C (SHORMANOV V K), 15 December 1994 (1994-12-15) abstract</p> <p>—</p> <p>WO 96 30376 A (CHEMFERM VOF.; BOESTEN WILHELMUS H J (NL); MOODY HAROLD MONRO (NL);) 3 October 1996 (1996-10-03) page 3, line 23-27; claims</p> <p>—</p> <p>EP 0 261 823 A (TANABE SEIYAKU CO) 30 March 1988 (1988-03-30) claims</p> <p>—</p> <p>—/—</p>	1-16
Y		1-16
A		1-16

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the International filing date
- *L* document which may throw doubts on priority, claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *D* document referring to an oral disclosure, use, exhibition or other means
- *T* document published prior to the International filing date but later than the priority date claimed

T later document published after the International filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

C document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

V document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

B document member of the same patent family

Date of the actual completion of the International search

Date of mailing of the International search report

20 July 1999

29/07/1999

Name and mailing address of the ISA

European Patent Office, P.O. 5018 Patentzaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tel. 31 651 ext. n.
Fax (+31-70) 340-3018

Authorized officer

Chouly, J

INTERNATIONAL SEARCH REPORT

Date of Application No

PCT/NL 99/00246

C. (Continued) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB 599 854 A (SHELL DEVELOPMENT CO.) 23 March 1948 (1948-03-23) page 3, line 24; claims	1-16
E	WO 99 24441 A (HEITMANN HERWARDT; VROON ERIK DE (NL); GIST BROCADES BV (NL); ROY) 20 May 1999 (1999-05-20) claims	1-16

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 99/00246

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
RU 2024851	C	15-12-1994	NONE	
WO 9630376	A	03-10-1996	BE 1009264 A AU 4958296 A CN 1185157 A JP 9703074 A EP 0869960 A JP 11502832 T	07-01-1997 16-10-1996 17-06-1998 14-01-1998 14-10-1998 09-03-1999
EP 0261823	A	30-03-1988	JP 1756798 C JP 4048795 B JP 63079888 A AT 389311 B AT 59655 T AU 592467 B AU 7882787 A CA 1286285 A CN 2021225 B DK 500887 A FI 874152 A,B GR 3001274 T HK 42093 A IE 60042 B PH 23644 A PT 85773 A,B SU 1517764 A US 4866170 A ZA 8706739 A	23-04-1993 07-08-1992 09-04-1988 27-11-1989 15-01-1991 11-01-1990 31-03-1988 16-07-1991 15-06-1993 25-03-1988 25-03-1988 25-08-1992 07-05-1993 18-05-1994 27-09-1989 01-10-1987 23-10-1989 12-09-1989 14-03-1988
GB 599854	A		BE 465649 A	
WO 9924441	A	20-05-1999	NONE	